



What is claimed is:

1. An expression cassette comprising,
a polynucleotide encoding *luxA*, *luxB*, *luxC*, *luxD* and *luxE* gene products,
5 wherein (a) transcription of the polynucleotide results in a polycistronic RNA encoding
all the gene products; (b) each of the *luxA*, *luxB*, *luxC*, *luxD* and *luxE* gene products is
expressed as an individual polypeptide; and (c) polynucleotide sequences comprising
Gram-positive ribosome-binding site sequences are located 5' to all of said *lux* coding
sequences.
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2. The expression cassette of claim 1, further comprising a multiple-insertion site
located 5' to said *luxA*, *luxB*, *luxC*, *luxD* and *luxE* coding sequences.
3. The expression cassette of claim 1, wherein at least one Gram-positive
15 ribosome binding site comprises the sequence presented as SEQ ID NO:1.
4. The expression cassette of claim 1, wherein the coding sequences of the gene
products are derived from *Photobacterium luminescens*.
- 20 5. The expression cassette of claim 1, wherein the polynucleotide further
comprises a promoter located 5' to all of said *lux* coding sequences wherein transcription
of the polynucleotide results in a polycistronic RNA encoding all the *lux* gene products.
6. The expression cassette of claim 5, wherein said promoter is contained in an
25 Expression Enhancing Sequence selected from the group consisting of Sa1, Sa2, Sa3,
Sa4, Sa5, and Sa6.
7. The expression cassette of claim 5, wherein said promoter is contained in an
Expression Enhancing Sequence selected from the group consisting of Sp1, Sp5, Sp6,
30 Sp9, Sp16 and Sp17.

8. The expression cassette of claim 7, wherein said promoter is contained in Expression Enhancing Sequence Sp16.

5 21. An expression cassette comprising,
a polynucleotide encoding *luxA*, *luxB*, and *luc* gene products, wherein (a) transcription of the polynucleotide results in a polycistronic RNA encoding all three gene products, (b) polynucleotide sequences comprising Gram-positive ribosome-binding site sequences are located adjacent the 5' end of the *luxA* coding sequences, adjacent the 5'
10 end of the *luxB* coding sequences, and adjacent the 5' end of the *luc* coding sequences, and (c) each of the *luxA*, *luxB*, and *luc* gene products is expressed as an individual polypeptide.

22. The expression cassette of claim 21, wherein said polynucleotide further
15 encodes *luxC*, *luxD* and *luxE* gene products, wherein (i) Gram-positive ribosome-binding site sequences are located 5' to each of the *luxC*, *luxD*, and *luxE* coding sequences, and (ii) each of the *luxC*, *luxD*, and *luxE* gene products is expressed as an individual polypeptide.

20 24. The expression cassette of claim 21, wherein the polynucleotide further comprises a promoter located 5' to all of said *lux* and *luc* coding sequences wherein transcription of the polynucleotide results in a polycistronic RNA encoding all the *lux* and *luc* gene products.

25 25. The expression cassette of claim 24, wherein said promoter is contained in an Expression Enhancing Sequence selected from the group consisting of Sa1, Sa2, Sa3, Sa4, Sa5, and Sa6.

26. The expression cassette of claim 24, wherein said promoter is contained in an Expression Enhancing Sequence selected from the group consisting of Sp1, Sp5, Sp6, Sp9, Sp16 and Sp17.

5 27. The expression cassette of claim 26, wherein said promoter is contained in Expression Enhancing Sequence Sp16.

28. The expression cassette of claim 21, further comprising a multiple-insertion site located 5' to said *luxA*, *luxB*, *luc*, *luxC*, *luxD* and *luxE* coding sequences.

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29. The expression cassette of claim 21, wherein the coding sequences for *luxA* and *luxB* are obtained from *Photorhadus luminescens*.

34. The expression cassette of claim 1, wherein the expression cassette is
15 contained within a bacterial transposon.

35. The expression cassette of claim 1, wherein the expression cassette is contained within a bacterial mini-transposon.

20 36. The expression cassette of claim 1, wherein the coding sequences of the gene products comprise codons that are optimal for expression of the gene products in a host system into which the expression cassette is to be introduced.

49. A shuttle vector comprising:
25 an expression cassette according to claim 1;
a polynucleotide encoding a selectable marker;
a Gram-positive origin of replication; and
a Gram-negative origin of replication.

56. A method of modifying a Gram-positive organism to produce light, comprising transforming the Gram-positive organism with an expression cassette according to claim 1.

5 58. A method of screening an analyte for its ability to affect expression of a reporter marker, comprising:

 providing the analyte to Gram-positive bacteria comprising the luciferase expression cassette of claim 1, wherein said reporter marker comprises luciferase; and

 monitoring the effect of the analyte on the ability of the Gram-positive bacteria to
10 produce light, thereby identifying whether the analyte affects expression of the reporter in Gram-positive bacteria.

64. A Gram-positive bacteria comprising an expression cassette according to claim 1.

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68. The expression cassette of claim 1, wherein the arrangement of the coding sequences for the *lux* gene products is in the following relative order 5' - *luxA-luxB-luxC-luxD-luxE*- 3'.

20 69. The expression cassette of claim 21, wherein the arrangement of the coding sequences for the *lux* gene products is in the following relative order 5' - *luxA-luxB-luxC-luxD-luxE*- 3'.

70. The expression cassette of claim 21, wherein the expression cassette is
25 contained within a bacterial transposon.

71. The expression cassette of claim 21, wherein the expression cassette is contained within a bacterial mini-transposon.

72. The expression cassette of claim 21, wherein the coding sequences of the gene products comprise codons that are optimal for expression of the gene products in a host system into which the expression cassette is to be introduced.

5 73. A shuttle vector comprising:
an expression cassette according to claim 21;
a polynucleotide encoding a selectable marker;
a Gram-positive origin of replication; and
a Gram-negative origin of replication.

10 74. A Gram-positive bacteria comprising an expression cassette according to claim 21.

15 75. A bacteria comprising the vector of claim 49.

76. A bacteria comprising the vector of claim 73.

20 77. A method of modifying a Gram-positive organism to produce light, comprising transforming the Gram-positive organism with an expression cassette according to claim 21.

78. The method of claim 77 further comprising providing the substrate required for *luc*-mediated luciferase activity.

25 79. A method of screening an analyte for its ability to affect expression of a reporter marker, comprising:
providing the analyte to Gram-positive bacteria comprising the luciferase expression cassette of claim 21, wherein said reporter marker comprises luciferase;
providing one or more substrates required for luciferase light production; and

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monitoring the effect of the analyte on the ability of the Gram-positive bacteria to produce light, thereby identifying whether the analyte affects expression of the reporter in Gram-positive bacteria.

80. The method of claim 79, wherein said one or more substrates comprise aldehyde provided as a vapor.

81. The method of claim 79, wherein said one or more substrates comprise a substrate for the *luc* gene product.

82. The method of claim 79, wherein said one or more substrates comprise (i) aldehyde, provided as a vapor, and (ii) a substrate for the *luc* gene product.